SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF BIS THIOUREA DERIVATIVES

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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF BIS THIOUREA DERIVATIVES

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Declaration

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning. I hereby declare that this project is the work of my own excluded for the references document and summaries that have been acknowledge.

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(AINAA NADIAH ABD HALIM)

Date:

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List of Abbreviations

FTIR	Fourier Transform infrared spectroscopy
^{13}C NMR spectroscopy	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR spectroscopy	Hydrogen Nuclear Magnetic Resonance
CHN analysis	Carbon Hydrogen Nitrogen analysis
MIC	Minimal Inhibitory Concentration
ppm	part per million
DMSO	Dimethyl sulfoxide
rpm	revolutions per minute

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Synthesis, Characterization and Antibacterial Activity of Bis Thiourea Derivatives

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ABSTRACT

A series of bis(thiourea) derivatives were successfully synthesized by reaction of benzene-1,4-dicarbonyl isothiocyanate intermediates, with amino acid and aromatic amine to afford thiourea **20-24** with overall yields of 11–58%. The structures of the synthesized compounds were characterized by infra red spectroscopy (FTIR), ^{13}C nuclear magnetic resonance (NMR), and ^{1}H NMR spectroscopy. The antibacterial activities of thiourea **20-24** were investigated on gram-negative bacteria, *Escherichia coli* using turbidimetric kinetic method. Minimum inhibition concentration (MIC) values of the compounds toward the bacteria were determined. The result obtained in this study indicated that only N1,N4-bis(phenylcarbamothioyl)terephthalamide **22** showed antibacterial activity against *E. coli* compared with other synthesized compounds.

Keyword: bis(thiourea) derivatives, Antibacterial activity, Amine, Spectroscopy

ABSTRAK

Satu siri terbitan bis(tiourea) derivatif telah berjaya dihasilkan melalui tindakbalas antara sebatian pertengahan benzena-1,4-dicarbonil isothiocianat, dan asid amino dan amina aromatik untuk menghasilkan thiourea **20-24** dengan hasil keseluruhan sebanyak 11-58%. Struktur sebatian yang telah disintesis dicirikan menggunakan spektroskopi infra merah (FTIR), resonans magnetik nuklear 13C (NMR), dan 1H NMR spektroskopi. Aktiviti antibakteria bagi thiourea **20-24** diuji ke atas bakteria gram-negatif, *Escherichia coli* menggunakan kaedah kinetik turbidimetrik. Nilai kepekatan rencatan minimum (MIC) sebatian ke atas bakteria tersebut dikaji. Hasil menunjukkan bahawa hanya N1, N4-bis (phenylcarbamothioyl) terephthalamide **22** menunjukkan aktiviti antibakteria terhadap *E. coli* berbanding dengan lain-lain sebatian yang disintesis.

Keyword: bis (tiourea) derivatif, aktiviti antibakteria, Amina, Spektroskopi

Chapter 1

Introduction

Thiourea is a compound which consists of sulphur and nitrogen and a chemical formula of CSN_2H_4. The basic structure of thiourea is shown in Figure 1 below. Thiourea has become intensely synthesized due to its ability to undergo structural modifications. Thiourea and its derivatives display a broad spectrum of applications in industries, chemistry, medicine and others (Ibrahim *et al.*, 2009). It is a unique compound having three different functional groups which are amino, imino and thiol and it can occur in tautomeric forms as shown in Figure 2. There is a lot of possible reactions that can lead to synthesis of new derivatives that may be applicable (Stuttgart, 1995).

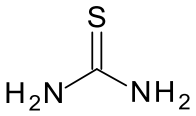
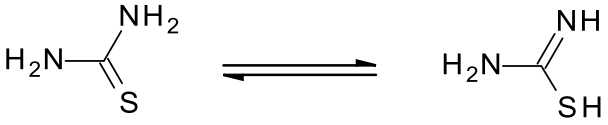


Figure 1: Thiourea



thiourea isothiourea

Figure 2: Tautomeric forms of thiourea

The common method in the synthesis of thiourea is by direct reaction of isothiocyanate with amine. This reaction involves nucleophilic attack at the electrophilic carbon of thiocyanate ion by amine (McEwen, 1991). This common method required the uses of solvent such as acetone, dimethylformamide, dichloromethane, and benzene (Todoulou *et al*., 1994, Cantrell *et al*., 1996, Wiles and Suprunchuk ,1969).

Thiourea derivatives are widely used in many fields including pharmaceutical industry due to their biological properties such as anticancer, antimicrobial, antibacterial, antifungal and antimalarial (Saeed *et al*., 2010, Saeed *et al*., 2009, Rauf *et al*., 2009, Campo *et al*., 2004, Solomon *et al*., 2010, Karakus & Rollas, 2002). Thiourea derivative has been reported to have anti-oxidant, anti-HIV, anti-tuberculosis agent and many other properties (Saturnino *et al*., 2003, Küçükgüzel *et al*., 2008).

In agricultural industry, thiourea has also widely used. Acyl thioureas, for example are known for their superior pesticidal, fungicidal, antiviral and regulating activity for plant growth (Rabea *et al*., 2009) while some have been shown to have notable positive effect on germination of mass seed as well as on chlorophyll contents in seedling leaves (Libe *et al*., 1994).

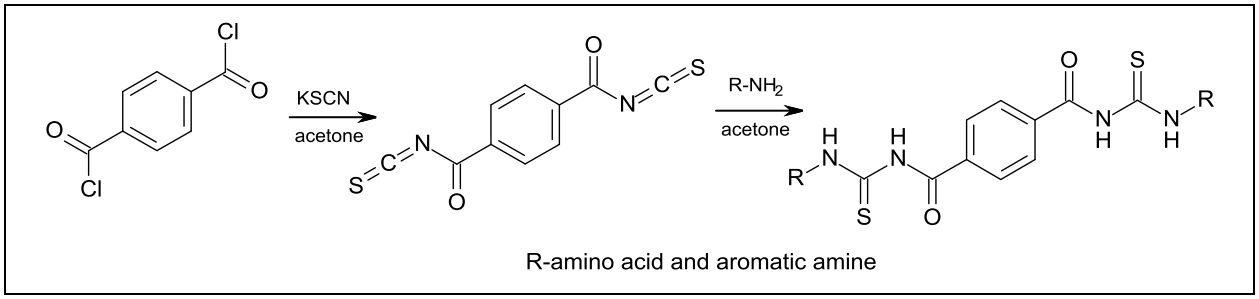
Due to numerous properties, thiourea becomes an important compound that has the ability to become a precursor in organic synthesis.

1.1 Problem Statement

Thiourea is very unique compound which consist of three functional groups such as amino, imino and thiol. Many studies on the synthesis of mono thiourea group reported on the good antibacterial activity (Mohammad *et al*., 2011). However, bis(thiourea) group are rarely reported. Therefore in this study, compound with two thiourea moieties were synthesized for possibilities of better antibacterial activities.

1.2 Objectives

1. To synthesize new bis(thiourea) derivatives as shown in Scheme 1.



Scheme 1: Reaction pathway of synthesis thiourea derivatives

2. To characterize bis(thiourea) derivatives using FTIR, ^{1}H NMR, ^{13}C NMR and CHN analysis.
3. To study the antibacterial activity of the synthesized bis(thiourea) derivatives against *Escherichia coli*.

Chapter 2

Literature Review

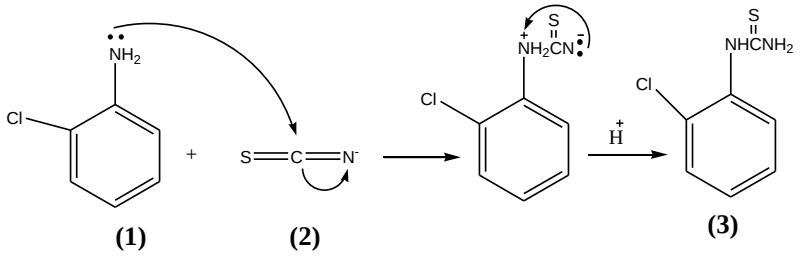
2.1 Thiourea

Thiourea or also known as thiocarbamide is a white crystalline solid compound with a chemical formula of CSN_2H_4 and molecular weight of 76.12 g/mol. Thiourea is soluble in water but insoluble in non-polar solvents. It is also soluble in polar protic and aprotic organic solvents such as acetone and dimethylsulfoxide (Stuttgart, 1995).

Thiourea is a type of heterocyclic compounds contains nitrogen and sulfur atoms which reported to exhibit a wide variety of biological activity **(**Khan *et al*., 2008**).** Several researches have reported thiourea derivatives as anti-oxidant, anti-HIV and anti-tuberculosis agent (Saturnino *et al*., 2003, Küçükgüzel *et al*., 2008). Thiourea derivatives are able to be adapted and used in many fields due to their biological properties such as anticancer, antimicrobial, antibacterial, antifungal and antimalarial (Saeed *et al*., 2010, Saeed *et al*., 2009, Rauf *et al*., 2009, Campo *et al*., 2004, Solomon *et al*., 2010, Karakus & Rollas, 2002).

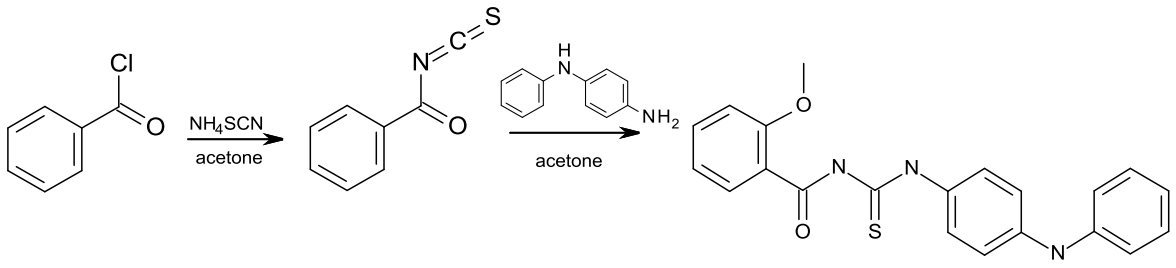
2.1.1 Synthesis of Thiourea derivatives

Thiourea derivatives **3** can be synthesized by direct reaction of isothiocyanate **2** with amine **1**. The reaction mechanism involved nucleophilic attack at the electrophilic carbon of thiocyanate ion by amine (McEwen, 1991). The general mechanism is shown in Scheme 2.



Scheme 2: General mechanism to synthesis thiourea

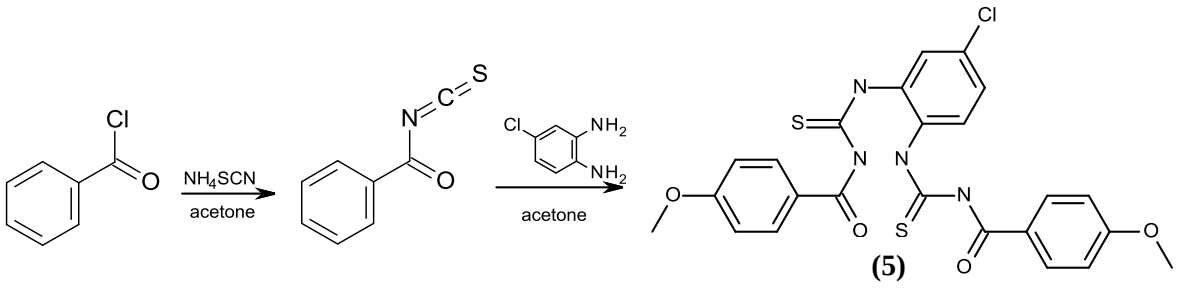
One of the example to synthesis thiourea is shown in Scheme 3. N-(2-methoxybenzoyl)-N'-(4-diphenylamine) thiourea was successfully synthesized by the reaction of benzoyl chloride with ammonium thiocyanate and N-phenyl-1,4-phenylenediamine in acetone as a solvent (Mohammad *et al*., 2011). The compound was studied for antibacterial activity and displayed excellent activity against *Staphylococcus aureus*. The present of methoxy and phenyl group substitution in the compound was reported to contribute to the biological activity.



(4)

Scheme 3: Reaction pathway of N-(2-methoxybenzoyl)-N'-(4-diphenylamine) thiourea 4

Thiourea was also reported as bis(thiourea). Bis(thiourea) is a thiourea compound with the present of two thiourea moieties and it was believed to increase antibacterial activities. For examples is the synthesized of 1,2-bis(N'-4-methoxybenzoylthioureido)-4-chlorobenzene **5** from reaction of benzoyl isothiocyanate with 4- chloro-1,2-phenylenediamine. The reaction was performed in acetone to give 69% yield of **5** (Mohammad *et al*., 2011). The compound was studied for antibacterial activity and show excellent activity against *Staphylococcus aureus* as well. The reaction pathway is shown in Scheme 4.

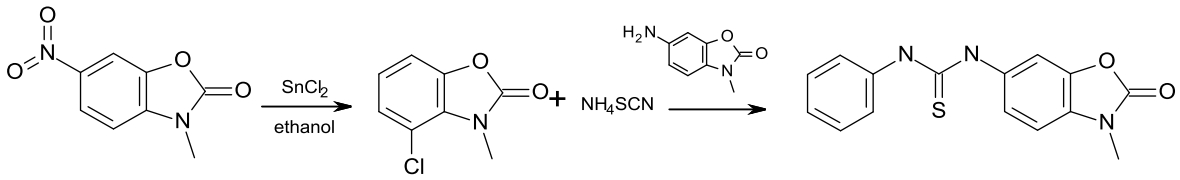


(5)

Scheme 4: Reaction pathway of 1,2-Bis[N'-(2-methoxybenzoyl)thioureido]-4-nitrobenzene

2.1.2 Solvent in synthesis of thiourea derivatives

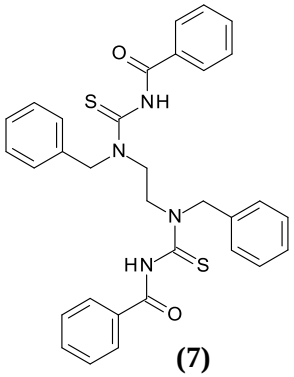
Solvent play a crucial roles in the synthesis of thiourea. Several types of solvent have been reported to be used in the synthesis of thiourea derivatives. For example, Yildiz *et al.,* (2012) using tetrahydrofuran (THF) as a solvent to synthesis 1-phenyl-3-(3-methyl-2-oxo-3H-benzoxazole-6-yl)thiourea **6**. Compound **6** was synthesized from the reaction of isothiocyanate derivatives with the solution of 6-amino-3-methyl-2(3H)-benzoxazolone. This compound **6** was successfully synthesized however the yield was only 17%. The reaction pathway is as in Scheme 5.



(6)

Scheme 5: Reaction pathway of 1-Phenyl-3-(3-methyl-2-oxo-3H-benzoxazole-6-yl)thiourea **6**

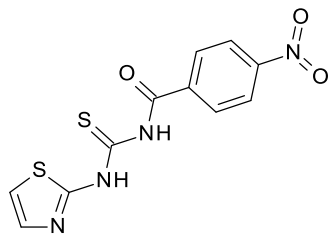
Benzene also been used as a solvent to synthesize thiourea derivatives. [N,N′-Bis(N-benzoylthiocarbamoyl)-N,N′-bis(benzyl)]ethane-1,2-diamine **7** was successfully synthesized from the reaction of benzoyl chloride with potassium thiocyanate and tetra-n-butylammonium bromide (TBAB) in the refluxing benzene to afford moderate 75% yield (Asha *et al.*, 2008).



(7)

Figure 3: [N,N′-Bis(N-benzoylthiocarbamoyl)-N,N′-bis(benzyl)]ethane-1,2-diamine **7**

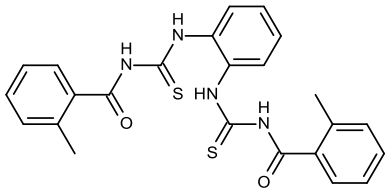
The most common solvent used in synthesized of thiourea derivatives was acetone. Saeed *et al*., (2009) has synthesized 1-(4-nitrobenzyl)-3-(thiazol-2-yl) thiourea **8** by the reaction of 4-nitrobenzoyl chloride and ammonium thiocyanate with tetrabuthyl ammonium bromide (TBAB) in refluxing acetone with the yield of 93%.



(8)

Figure 4: 1-(4-nitrobenzyl)-3-(thiazol-2-yl) thiourea **8**

Uwaisulqarni and Bohari, (2012) also has widely used acetone as solvent in their studies. They have successfully synthesized 1,2-bis(N'-2-methyl-benzoylthioureido) benzene **9** from the reaction of 2-methylbenzoylchloride and ammonium thiocyanate with 1,2-phenylenediamine in acetone. Compound **9** was produced in more than 90% yield.



(9)

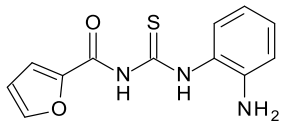
Figure 5: 1,2-bis(N'-2-methyl-benzoylthioureido) benzene **9**

Acetone is commonly used as a solvent to synthesize thiourea and their derivatives and produce higher yield compared to other solvent such as THF and benzene.

2.2 Biological properties of thiourea derivatives

2.2.1 Thiourea derivatives with anti-intestinal nematode treatment.

1-(2'-Furanyl)acyl-3-(2'-aminophenyl)thiourea **10** was synthesized and reported to have useful lead compounds for anti-intestinal nematode treatment (Li Ping Duan *et al.,* 2010). Compound **10** has the highest activity of against *Nippostrongylus brazilliensis* with 89.4 % deparasitization. This is due to the presence of furanyl moiety which seemed to be much more effective in terms of anti-intestinal nematode activity (Li Ping Duan *et al.,* 2010).



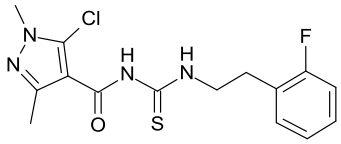
(10)

Figure 6: Compound 8 with anti-intestinal nematode treatment **10**

2.2.2 Thiourea derivatives with antifugal activity

In agricultural field, acyl thioureas are well known for their superior pesticidal, fungicidal, antiviral and regulating activity for plant growth (Rabea *et al*., 2009). Some thiourea have been shown to have notable positive effect on germination of mass seed as well as on chlorophyll contents in seedling leave (Libe *et al*., 1994).

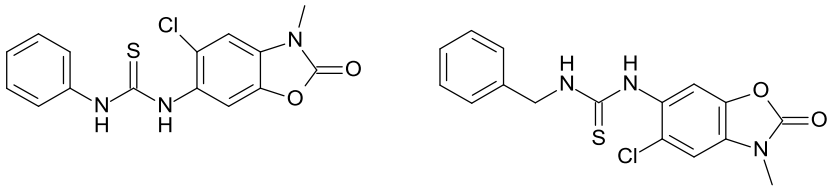
Wu *et al.,* (2012) reported on 1-(2-Fluorophenethyl)-3-(5-chloro-1,3-dimethyl-1H-pyrazole-4-carbonyl)thiourea **11** which exhibited similar activity on the corresponding fungi as compared to the commercial hymexazol. Compound **11** which prepared from thionyl chloride and ammonium thiocyanate with fluorinated aromatic amine showed antifungal activity against *Gibberella zeae*, *Fusarium oxysporum* and *Cytospora mandshurica* with the inhibition growth of 48.6%, 57.9% and 59.7% respectively.



(11)

Figure 7: Compound **11** with anti-fungal activity

Other examples are are 1-phenyl-3-(5-chloro-3-methyl-2-oxo-3H-benzoxazole-6-yl)thiourea **12** and 1-Benzyl-3-(5-chloro-3-methyl-2-oxo-3H-benzoxazole-6-yl)thiourea **13**. These thiourea derivatives, exhibited a relatively good inhibitory profile against *Escherichia coli*, with a MIC value of 32 μg/mL. Both compound **12** and **13** were also displayed good activity against *Candida albicans* with minimum inhibition concentration (MIC) value of 64 μg/mL (Yildiz *et al.,* 2012).



(12) **(13)**

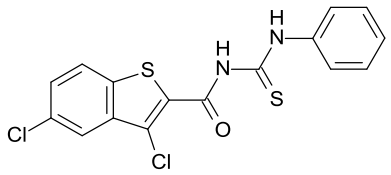
Figure 8 : Compound **12** and **13** with antifungal activity

Studies by Plech *et al*.,(2011), have reported that the introduction of halogen atoms to the pharmacophore structure can be beneficial for antimicrobial activity. The higher activity in these compounds **12** and **13** might be attributed to chloro substituent in the compound.

2.2.3 Thiourea derivatives with antibacterial activity

Antibacterial studies of monothiourea derivative are progressing at considerable rate. Some monothiourea derivatives are screened for their antibacterial activity against some of the pathogenic bacteria like *Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424), *Staphylococcus aureus* (MTCC96) and *Streptococcus pyogenes* (MTCC443).

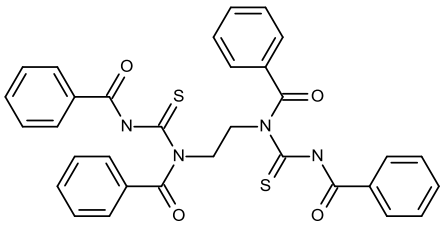
1-(3,5-dichlorobenzo[b]thiophene-2-carbonyl)-3-phenylthiourea **14** was synthesized by the reaction of 3,5-dichlorobenzo[b]thiophene-2-carbonylchloride and ammonium thiocyanate with aniline (Thakar *et al*., 2005). Compound **14** were active against *Staphylococcus aureus* and *Aspergillus niger* (Thakar *et al*., 2005).



(14)

Figure 9: Compound **14** with antibacterial activity

With the potential on antibacterial activity shown by monothiourea compound, bis(thiourea) has also recently received much attention. In 2008, Asha *et al*., was synthesized [N,N'-Bis(N-benzoylthiocarbamoyl)-N,N'-bis(benzoyl)ethane-1,2-diamine] **15** and was reported to show moderate antibacterial activity against 9 bacteria strains: *Serrana marcescens, Escherichia coli, Klebsiella preumoniae, Protieus vulgaris, Salomonella typhi, Psedomonas aerugenosa, Pseudomonas flurescens, Staphylococcus aureus* and *Bacillus subtilis*.



(15)

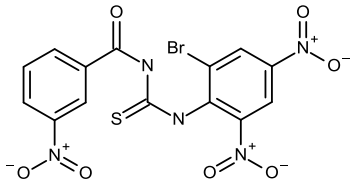
Figure 10: [N,N'-Bis(N-benzoylthiocarbamoyl)-N,N'-bis(benzoyl)ethane-1,2-diamine] **15**

Many studies on bis(thiourea) derivatives showed far better antibacterial activity against various bacteria strains compare to monothiourea compound.

2.2.3.1 The effect of substituent group in thiourea

The presence of substituent group such as nitro, chloro and bromo on thiourea has been reported to enhance the antibacterial activity of the compounds toward methicillin resistant *Staphylococcus aureu*s (Ibrahim *et al*., 2009).

N-(3-nitrobenzoyl)-N'-(2-bromo-4,6-dinitrophenyl)thiourea **16** and N-pentanoyl-N'-(2-chloro-4,6-dinitrophenyl)thiourea **17**, for instance, have been reported to have antibacterial activity on *Staphylococcus aureu*s (Ibrahim *et al*., 2009).



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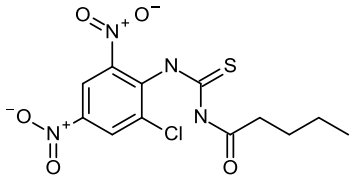


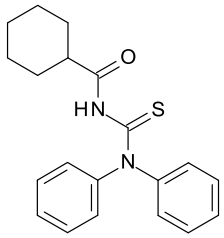
(17)

Figure 11: Compound **15** and **16** with antibacterial activity

The compound **16** inhibition two times higher in concentration compare to compound **17** (Ibrahim *et al*., 2009). This may due to the difference in reactivity between Br and Cl. Varied effects may also due to bulkiness of the side chain because bulky group in the structure cause the occurrence of steric hindrance which prevents the compounds from reaching the active site (Fernandez *et al*., 2005).

Presence of the cyclohexyl moiety or other derivatives included substituted benzyl groups may also affect the activity of the compound against bacteria. Arslan *et al*., (2009) has synthesized N-(diphenylcarbamothioyl) cyclohexanecarboxamide **18** that showed high activity against all gram-positive bacterias: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus cereus*.

Lipophilicity, which measure with the n-octanol/water partition coefficient (log *P*ow) is correlates well with the bioactivity of chemicals and it is a very important molecular descriptor. Compounds with benzyl groups have relatively higher log *P*ow values and hence show more lipophilic character as compared to the compounds with cyclohexyl groups (Hoey *et al*., 1996). Compound **18** has two benzyl group substituents show higher lipophilicity character, thus these compound can penetrate into the microorganisms easily.



(18)

Figure 12: Compound **18** with antibacterial activity